

Exhibit A

Product specification

FluoroLink™-Ab Cy3 labelling kit PA 33000

Reagent kit for the conjugation of proteins with CyTM 3 reactive dye

Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Warning: Contains sodium azide. See safety data sheet supplied.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Storage

Store at 2-8°C in the dark. Do not use if desiccant capsule in foil pack is not blue.

Introduction

Cyanine reagents have been shown to be useful as fluorescent labels for biological compound^(1,2). These dyes are both water soluble and highly fluorescent, providing significant advantages over other existing fluorescent labels.

The Cy3 dye is an orange fluorescing cyanine that produces an intense signal easily detected using most rhodamine filter sets. The Cy3 dye supplied here is a bisfunctional NHS-ester and is provided in a dried, pre-measured form ready for the labelling of compounds containing free amino groups.

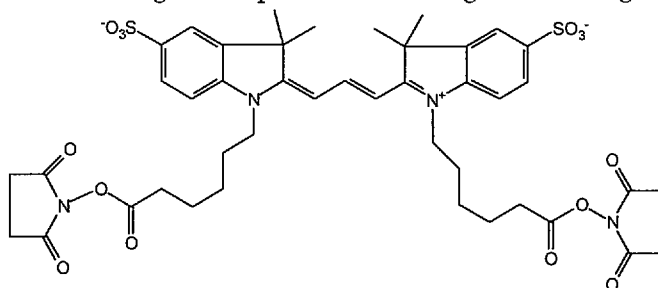


Figure 1. Cy3 bisfunctional dye

Materials supplied

- Two foil packs each containing dried dye to label 1mg of protein
- Coupling buffer (2 vials) [1M sodium carbonate buffer, pH9.3]
- Gel filtration column for the separation of labelled protein from non-conjugated dye
- Elution buffer (50ml) for the elution of labelled protein from the column (phosphate buffered saline, pH7.2, containing 0.1% sodium azide as a preservative. See safety data sheet supplied)
- Sample collection tubes (4)
- Transfer pipettes (6)
- Product specification sheet with directions for using the dye
- Safety data sheet

Materials required but not supplied

- Antibody to be labelled as 1mg total protein in 1ml of 50mM phosphate buffer, pH7.0-7.3, such as PBS

Recommended procedure for use

The protocol has been designed for the preparation of Cy3-labelled IgG antibodies. It is designed to label 1mg protein, to a final molar dye/protein (D/P) ratio between 4 and 12. This assumes an average protein molecular weight of 155000 daltons.

NOTE: The following materials and procedures have been optimised for IgG antibodies. Other antibodies may also be readily labelled; however, separation media and techniques may vary in order to produce optimum results.

Altering the protein concentration and reaction pH will change the labelling efficiency of the reaction.

Optimal labelling generally occurs at pH9.3. Proteins have been successively labelled with this dye at a pH as low as 7.3; however, labelling times must be significantly longer at lower pH. Higher protein concentrations usually increase labelling efficiency. Solutions of up to 10mg/ml protein have produced good conjugation reactions.

Conjugation of dye to antibody

Antibody to be conjugated should be dissolved at 1mg/ml in 50mM phosphate buffer (pH7.0-7.3) such as PBS⁽²⁾. Acceptable labelling may be obtained with a protein range of 0.5mg to 1.5mg. A smaller amount of protein is likely to result in over-labelling (possibly inactivated antibodies, appearance of non-specific binding, and reduced fluorescence quantum yield). A larger amount of protein will result in under-labelled antibodies that are not optimally bright.

If the antibody is in carrier protein, hybridoma supernatant, or ascites fluid, it is still possible to label it. However, any other protein in the solution will also be labelled. It is essential that all non-antibody protein present in the sample be counted in the 1mg protein that is labelled by this procedure. In test applications, the presence of the Cy3 label on these other proteins has been found not to interfere with immunofluorescence experiments provided that these proteins did not bind non-specifically to the sample material.

NOTE: Buffers containing primary amino groups such as TRIS and glycine will inhibit the conjugation reaction. The presence of low concentrations (<2%) of biocides such as azide or thimerosal do not affect protein labelling.

1. Add the protein solution (1ml) to the vial of coupling buffer and mix thoroughly by gentle vortexing or by manually inverting the capped tube 10 times.

CAUTION: This dye is intensely coloured and very reactive. Care should be exercised when handling the dye vial to avoid staining clothing, skin and other items.

2. Transfer the entire volume of protein and coupling buffer to the vial of reactive dye, cap the vial and mix thoroughly. Care should be taken to prevent foaming of the protein solution. The reaction is incubated at room temperature for 30 minutes with additional mixing approximately every 10 minutes.

Separation of protein from free dye

1. While the labelling reaction is incubating, decant the buffer from the top of the column. Mount the column on a ring stand.

2. Add 13ml fresh elution buffer. Remove the tip from the column to start the outflow of the column and allow all the buffer to run through the column into a collection tube or small beaker. Flow will automatically stop when the meniscus reaches the disk at the top of the column packing. There is no need to worry about the column drying out.

3. Carefully transfer the antibody-labelling mixture to the top of the column and allow the solution to enter the packing.

4. Add 2.0ml of elution buffer. As this volume of buffer moves through the column, a faster moving pink band of labelled protein will separate from the unconjugated dye.

5. When the 2.0ml of elution buffer has completely run into the column packing, the leading edge of the faster moving pink band should be near the bottom of the packing.

6. Add an additional 2.5ml of elution buffer to the top of the column and collect the faster moving pink band in a clean tube as it elutes from the column. The labelled protein should be entirely eluted by the 2.5ml of buffer and collected in a single tube.

Notes

- The labelled protein solution contains 0.1% sodium azide as a preservative. It should be protected from excess light and stored at 2-8°C.
- The remaining free dye can be removed from the column (prior to additional separations) with 13ml of the elution buffer solution.

- Unconjugated dye can also be separated from the labelled antibody by dialysis. Dialysis does not give as efficient and rapid separation as gel filtration. We therefore recommend that gel filtration be used whenever possible.

Estimation of final dye/protein (D/P) ratio

Dilute a portion of the labelled protein solution so that the maximum absorbance is 0.5 to 1.5AU. Molar concentrations of dye and protein are calculated, and the ratio of these values is the average number of dye molecules coupled to each protein molecule. Molar extinction coefficients of $150000 \text{ M}^{-1}\text{cm}^{-1}$ at 552nm for the Cy3 dye and $170000 \text{ M}^{-1}\text{cm}^{-1}$ at 280nm for the protein are used in this example. The extinction coefficient will vary for different proteins. The calculation is corrected for the absorbance of the dye at 280nm (approximately 8% of the absorbance at 552nm).

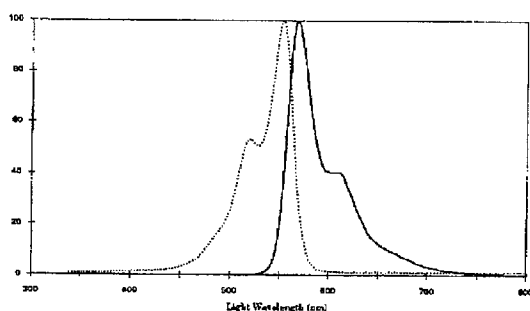
$$[\text{Cy3 dye}] = (A_{552}) / 150000$$

$$[\text{antibody}] = \{A_{280} - (0.08 \cdot A_{552})\} / 170000$$

$$(\text{D/P})_{\text{final}} = [\text{dye}] / [\text{antibody}]$$

$$(\text{D/P})_{\text{final}} = \{1.13 \cdot (A_{552})\} / \{A_{280} - (0.08 \cdot A_{552})\}$$

Figure 2. Cy3 dye absorption and fluorescence spectra



Cy3 bisfunctional dye characteristics

Formula weight	949.11
Absorbance max	550nm
Extinction max	$150000 \text{ M}^{-1}\text{cm}^{-1}$
Emission max	570nm
Quantum yield	>0.15*
* for labelled proteins, D/P =2	

References

1. MUJUMDAR, R.B. *et al.*, *Bioconjugate Chemistry*, 4 (2), pp.105-111, 1993.
2. SOUTHWICK, P.L. *et al.*, *Cytometry*, 11, pp.418-430, 1990.

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PA33000PS/AB

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FluoroLink-Ab

Cy5 labelling kit

PA 35000

Reagent kit for the conjugation of proteins with CyTM5 reactive dye

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Introduction

Cyanine reagents have been shown to be useful as fluorescent labels for biological compound^(1,2). These dyes are both water soluble and highly fluorescent, providing significant advantages over other existing fluorescent labels.

Cy5 dye produces an intense signal in the far-red region of the spectrum. Though not recommended for visual applications, this dye is ideally suited for detection using CCD cameras, PMTs and some red-sensitive films. The Cy5 dye supplied here is a bisfunctional NHS-ester and is provided in a dried, pre-measured form ready for the labelling of compounds containing free amino groups.

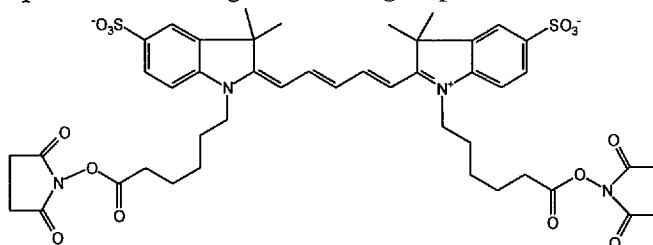


Figure 1. Cy5 bisfunctional dye

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- Two foil packs each containing dried dye to label 1mg of protein
- Coupling buffer (2 vials) [1M sodium carbonate buffer, pH9.3]
- Gel filtration column for the separation of labelled protein from non-conjugated dye
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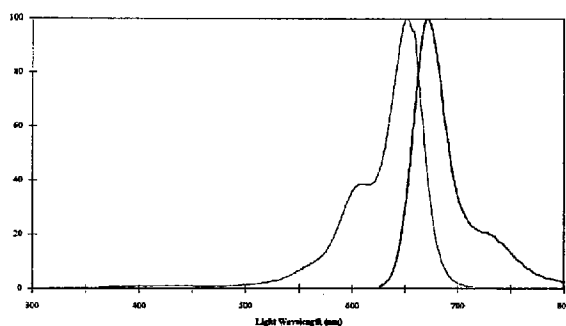
$$[\text{Cy5 dye}] = (A_{650}) / 250000$$

$$[\text{antibody}] = \{A_{280} - (0.05 \cdot A_{650})\} / 170000$$

$$(\text{D/P}) \text{ final} = [\text{dye}] / [\text{antibody}]$$

$$(\text{D/P}) \text{ final} = \{0.68 \cdot (A_{650})\} / \{A_{280} - (0.05 \cdot A_{650})\}$$

Figure 2. Cy5 dye absorption and fluorescence spectra



Cy5 bisfunctional dye characteristics

Formula weight	975.15
Absorbance max	649nm
Extinction max	$250000 \text{ M}^{-1}\text{cm}^{-1}$
Emission max	670nm
Quantum yield	$>0.28^*$

* for labelled proteins, D/P =2

References

1. MUJUMDAR, R.B. *et al.*, *Bioconjugate Chemistry*, 4 (2), pp.105-111, 1993.
2. SOUTHWICK, P.L. *et al.*, *Cytometry*, 11, pp.418-430, 1990.

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